JOURNAL OF CHEMICAL EDUCATION

Communication

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Extraction of Eugenol from Cloves Using an Unmodified Household Espresso Machine: An Alternative to Traditional Steam-Distillation

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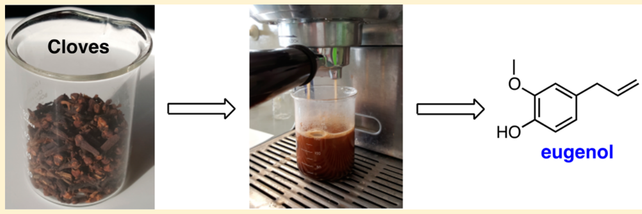
Supporting Information

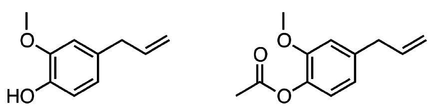
ABSTRACT: This laboratory experiment describes the extraction of natural products from plant material in line with recent research advances in this field. Specifically, an unmodified household espresso machine is used to extract the essential oil components of cloves (eugenol and acetyleugenol). This straightforward and uncomplicated method expedites the extraction of clove oil and requires simple and relatively inexpensive equipment. Moreover, this technique is successfully used to replace the steam-distillation of cloves experiment that has traditionally been part of a second-year undergraduate laboratory program.



KEYWORDS: *Second-Year Undergraduate, Natural Products, Organic Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning*

INTRODUCTION

Attempting to incorporate relevant research developments in undergraduate curricula, and laboratory programs, in particular, is an important and challenging aspect of chemical education, and scientific education, more generally.[1] With this in mind, a recently reported,[2] straightforward method for rapid extraction of natural products using an unmodified household espresso machine presented an excellent opportunity to incorporate significant outcomes from the research laboratory directly into undergraduate teaching. To this end, an existing and well-established second-year organic chemistry laboratory experiment that involves the isolation of eugenol (**1**) from cloves (Figure 1) by steam-distillation has been modified.[3]



(**1**) (**2**)

Figure 1. Primary components of clove oil: eugenol (**1**) and acetyleugenol (**2**).

The extraction and isolation of organic molecules from plant material can be used to reinforce a range of important fundamental principles, including spectroscopy,[4] chromatography,[5] and aspects of medicinal chemistry.[6] In an undergraduate environment, steam-distillation represents a technique that is introduced to chemistry students as a method for the isolation of compounds based upon their vapor pressure.[3,7,8] These experiments are relatively simple to undertake; however, the distillation itself can be a time-consuming process. In addition, students (or groups of students) require their own sets of equipment. The novel method for the isolation of eugenol from cloves using an espresso machine can be successfully completed in less time than the traditional steam-distillation procedure. In addition, students are able to share this equipment in order to perform the extraction as this process takes ~2 min per sample. This minimizes the need for expensive items of equipment, including distillation apparatus and hot plates/heating mantles. The time students spend waiting in the extraction step is minimized in this experiment, and this procedure is more cost-effective and time-efficient. Moreover, as part of a first-year chemistry curriculum, students are required to put together and subsequently use standard distillation equipment, and it is important to maximize undergraduates' exposure to new techniques in order to complement their laboratory training where possible. Indeed, this experiment contributes to these aims.

Pressurized hot water extraction (PHWE) is a technique typically used in analytical chemistry where water is passed through a sample at high pressure.[9] These high pressures can enable water to be heated above its boiling point, if required. An espresso machine employs a similar principle, with hot water (93 °C) passed through a packed sample of ground coffee at pressures of ~9 bar.[10] It is reported that the extraction process for making coffee is based upon four fundamental principles: (i) the dissolution of soluble compounds from the sample (coffee grinds); (ii) the release of less soluble sample components into water facilitated by pressure; (iii) the thermal degradation of sample components resulting in improved solubility; and (iv) the presence of a mobile phase (water) to

DOI: 10.1021/acs.jchemed.5b00476
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remove compounds physically from the sample.[11] To utilize an espresso machine to effect the extraction of eugenol from cloves, it is likely that the last three processes are the most important in enabling an efficient extraction to occur.

In this experiment, students use an unmodified household espresso machine to extract the essential oil component of cloves: eugenol (**1**) and acetyleugenol (**2**) (Figure 1). The clove oil extract obtained in this way is subjected to a simple, acid–base extraction to enable the separation of eugenol from acetyleugenol. Students perform IR analysis of their eugenol sample. GC analysis is performed as part of this procedure; however, this is undertaken by lab technicians. ^{1}H and ^{13}C NMR spectra of eugenol are also provided to students. This experiment has been successfully implemented in a second-year undergraduate organic chemistry laboratory program.

■ EXPERIMENT

Students work individually and one, 4-h laboratory period is required to complete the experiment.

Sample Preparation

Cloves (store-bought) are ground (~10 s) in a spice/coffee bean grinder. This ground material (15 g) is mixed with acid-washed sand (2 g) to aid packing and to avoid clogging the sample compartment (portafilter) of an espresso machine.

Extraction Using an Espresso Machine

The extraction solvent (25% v/v ethanol/water) is poured into the solvent reservoir of an espresso machine and the machine is warmed to its operating temperature (90–95 °C). The mixture of ground cloves and sand is packed into the portafilter using a coffee tamper. The portafilter is fitted to the espresso machine, and the solvent is passed through the sample (~100 mL) and collected in a 250 mL beaker. After 0.5 min, more solvent is passed through the sample (~100 mL) and collected (~200 mL total extract volume). Because ethanol can cause emulsions during the solvent extraction process, it is removed by rotary evaporation (~10 min at full vacuum; water bath maintained at 50 °C).

Solvent Extraction

The ensuing aqueous phase is extracted with dichloromethane (3 × 50 mL). The combined dichloromethane phases are extracted with sodium hydroxide (3 × 30 mL of a 2 M aqueous solution) to extract eugenol selectively into the aqueous phase. The combined sodium hydroxide extracts are acidified with hydrochloric acid (10 M aqueous solution) until the pH of the mixture reaches 1 (an emulsion is observed to form; the pH can be tested with universal indicator paper). The ensuing aqueous mixture is extracted with dichloromethane (3 × 20 mL). The combined dichloromethane extracts are dried (magnesium sulfate), filtered, and concentrated using a rotary evaporator to provide eugenol (**1**).

It should be noted that dichloromethane can be substituted with the less toxic heptane in the solvent extraction step and comparable results are obtained. If heptane is used as the extraction solvent, the rotary evaporation of ethanol prior to the solvent extraction step is not required as emulsions are not as problematic in this case. An alternative procedure employing heptane as the extraction solvent is included in the Supporting Information. A range of solvents have been used for the solvent extraction of eugenol following the traditional steam-distillation of cloves.[3]

Analysis

Students analyze their eugenol samples by IR spectroscopy and prepare gas chromatography (GC) samples of the eugenol they have isolated. A lab technician performs GC analysis of these samples, and students are provided with their respective chromatograms. Students are provided with a ^{1}H NMR spectrum and a GC chromatogram of clove oil (containing both eugenol and acetyleugenol), and ^{1}H and ^{13}C NMR spectra and a mass spectrum of eugenol (see Supporting Information).

■ HAZARDS

This experiment should be undertaken in a fume hood, and students should wear all standard personal protective equipment in the laboratory (lab coat, safety glasses and gloves). Clove oil (which contains compounds **1** and **2**) is an irritant and care should be taken to avoid skin contact or inhalation. Dichloromethane is toxic, a suspected carcinogen, and a skin, eye, and lung irritant. Sodium hydroxide (2 M aqueous solution) and hydrochloric acid (10 M aqueous solution) are caustic and corrosive. Ethanol is flammable, and should be diluted (25% v/v in water) to make a less flammable solution prior to use in the coffee machine. The espresso machine operates at temperatures of ~90–95 °C. Dichloromethane should be transferred to the appropriate solvent waste container (see Supporting Information for further details).

■ RESULTS AND DISCUSSION

Within the context of the undergraduate chemistry program, this experiment was designed to highlight the strong link between natural products and organic chemistry by exposing students to a recently reported method for natural product extraction. Laboratory techniques, such as solvent extraction, including isolation of acidic compounds by acid–base extraction, and use of rotary evaporators, were reinforced/introduced. The experiment reinforced lecture material and, specifically, the role of spectroscopy in the elucidation of chemical structures. The assessment tasks that were incorporated in the laboratory report were focused on the structural assignment of eugenol by ^{1}H NMR, ^{13}C NMR and IR spectroscopy and mass spectrometry.

Three laboratory sections of 12–15 second-year undergraduate students (40 in total) performed this experiment. Students worked individually and comfortably completed this experiment within the allocated 4-h period. In general, students were readily able to carry out this procedure with minimal instruction and used this process to isolate up to ~0.5 g of eugenol from 15 g of cloves. Some students obtained only trace amounts of eugenol, which was attributed to errors during the acid–base extraction step. If students deviated from the prescribed experimental procedure during the acid–base extraction, this generally led to mistakes occurring during one of the three stages in this process and the eugenol-containing fraction was inadvertently discarded. However, this issue was not directly related to the new extraction method using the espresso machine. Efforts were made to minimize bottlenecks during the course of this experiment: two espresso machines were used during each laboratory session and four rotary evaporators were available for use.

For practical reasons, students were provided with ^{1}H and ^{13}C NMR spectra of eugenol and a ^{1}H NMR spectrum and GC chromatogram of clove oil (containing both eugenol and acetyleugenol) for analysis. Students prepared GC samples

DOI: 10.1021/acs.jchemed.5b00476
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from their isolated sample in order to determine the purity of their eugenol sample and also to search for the presence of acetyl eugenol in the sample. GC analysis was subsequently performed by a laboratory technician, and the data were provided to students. Students also analyzed their isolated eugenol sample by IR spectroscopy.

The results obtained by students in this experiment were consistent with results obtained by their counterparts that performed the traditional steam-distillation of cloves in previous years. Specifically, GC analysis indicated that the purity of eugenol samples isolated using this new procedure was very similar to the purity of samples obtained via the steam-distillation method. Furthermore, the yield of eugenol isolated by students typically ranged from 6 to 10% (w/w) using this espresso machine extraction method. This is consistent with results (7−9% w/w) obtained by undergraduates performing the traditional steam-distillation of cloves experiments reported in the literature.[3a] From the GC chromatograms of their samples (Supporting Information), students concluded that the eugenol isolated was free from acetyleugenol by comparison to the chromatogram of a sample of crude clove oil provided to them. In general, the purity of the students' isolated eugenol was very high. In the analysis of the ^{1}H and ^{13}C NMR spectra provided for eugenol to students (Supporting Information), they successfully assigned the aromatic, olefin, methoxy and methylene signals and determined the coupling constants were appropriate. In the IR spectra (Supporting Information), students focused on the − OH stretch for the phenolic group of eugenol and the absence of a carbonyl stretch that is observed for acetyl eugenol. In the mass spectrum (Supporting Information), students identified the molecular ion for eugenol.

In general, the feedback from students indicated that this experiment was very well-received and students were particularly intrigued by the use of a simple, household device to facilitate natural products extraction. Certainly, exposing undergraduates to interesting and innovative experimental techniques contributes to establishing an enjoyable, memorable, and ultimately successful laboratory experience for students.

This method for the extraction of eugenol from cloves using an espresso machine can be applied to facilitate the isolation of other natural products from plant material. Namely, shikimic acid from star anise and polygodial from *Tasmannia lanceolata* (Tasmanian native pepper).[2,12] In addition, preliminary experiments undertaken by undergraduates in the laboratory indicated that carvone can be extracted from caraway seeds, thymol can be isolated from either thyme or oregano, and caffeine can be extracted from tea leaves by this approach.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00476.

NMR spectra, spectral data, laboratory manual procedure, laboratory demonstrator notes (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the University of Tasmania for a Teaching Development Grant and the Central Science Laboratory at University of Tasmania for access to NMR spectroscopy services. The authors thank Brendon Schollum for performing GC analysis of eugenol samples. The authors thank reviewers for their helpful suggestions and comments.

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DOI: 10.1021/acs.jchemed.5b00476
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